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In Vitro Activity of Ferroquine Is Independent of Polymorphisms in Transport Protein Genes Implicated in Quinoline Resistance in *Plasmodium falciparum*▽

Maud Henry,[1,2] Sébastien Briolant,[1,2] Albin Fontaine,[1,2] Joel Mosnier,[1,2] Eric Baret,[1,2] Rémy Amalvict,[1,2] Thierry Fusaï,[1,2] Laurent Fraisse,[3] Christophe Rogier,[1,2] and Bruno Pradines[1,2]*

Unité de Recherche en Biologie et Epidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France[1]; Unité de Recherche pour les Maladies Infectieuses et Tropicales Emergentes, Unité Mixte de Recherche 6236, Marseille, France[2]; and Sanofi-Aventis Recherche et Développement, Sanofi-Aventis, Toulouse, France[3]

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The in vitro activity of ferroquine (FQ) (SR97193), a 4-aminoquinoline antimalarial compound that contains a ferrocenic nucleus, against 15 *Plasmodium falciparum* strains was assessed and compared with those of chloroquine (CQ), quinine (QN), monodesethylamodiaquine (MDAQ), and mefloquine (MQ). These 15 strains were genotyped for polymorphisms in quinoline resistance-associated genes such as Pf*crt*, Pf*mdr1*, Pf*mrp*, and Pf*nhe-1*. FQ was highly active against CQ-resistant parasites or in parasites with reduced susceptibility to QN, MDAQ, or MQ. Encouragingly, we did not find a correlation between responses to FQ and those to other quinoline drugs. These results suggest that no cross-resistance exits between FQ and CQ or quinoline antimalarial drugs. Mutations in codons 74, 75, 76, 220, 271, 326, 356, and 371 of the Pf*crt* gene; codons 86, 184, 1034, 1042, and 1246 of the Pf*mdr1* gene; and codons 191 and 437 of the Pf*mrp* gene were not significantly associated with *P. falciparum* susceptibility to FQ. Neither the number of ms4760 DNNND or DDNHNDNHNN repeats in Pf*nhe-1* nor the profile of ms4760 was significantly associated with the FQ in vitro response. These data suggest the FQ may not interact with transport proteins in quinoline-resistant parasites. The present results justify further clinical trials of FQ in multidrug resistance areas.

Two of the current options to reduce the morbidity and mortality of malaria are chemoprophylaxis and chemotherapy. During the past 20 years, many strains of *Plasmodium falciparum* have become resistant to chloroquine and other antimalarial drugs (24). This has prompted a search for an effective alternative antimalarial drug with minimal side effects. The emergence and spread of parasites that are resistant to antimalarial drugs has caused an urgent need for novel compounds to be discovered and developed.

An approach to remove aminoquinoline resistance in parasites is to modify the position and the chemical nature of the substituents or the length of the side chain on the quinoline nucleus of the aminoquinoline (12, 34). Recently, many different metals have been incorporated into antimalarial agents (29). Indeed, several organometallic compounds based on chloroquine with a ferrocene nucleus localized at different sites have been synthesized (5–8). This approach is currently being developed by J. Brocard and colleagues (URA-CNRS 402, Lille, France), who have synthesized ferroquine (FQ) {i.e., 7-chloro-4-[(2-*N*,*N*′-dimethylaminomethyl)ferrocenylmethylamino]quinoline} (Fig. 1). FQ is currently under phase II clinical trial investigations.

Only six previous studies investigated the activity of ferroquine against *P. falciparum* strains isolated from infected patients (1, 2, 10, 21, 28, 30). The drug susceptibilities of *P. falciparum* strains vary among different locations, where isolates have different antimalarial resistance backgrounds. It seems that ferroquine activity is independent of chloroquine resistance in *P. falciparum* (21), and ferroquine antimalarial activity is not influenced by polymorphisms in the Pf*crt* gene (*Plasmodium falciparum* chloroquine resistance transporter), which encodes a protein located in the parasite digestive vacuole and is involved in drug transport and chloroquine resistance (10, 11).

The objective of this study was to determine whether genetic polymorphisms in genes associated with quinoline resistance modulate in vitro responses to ferroquine. We assessed polymorphisms in genes that are potentially associated with quinoline resistance: Pf*crt*, Pf*mdr1* (*P. falciparum* multidrug resistance gene 1), Pf*nhe-1* (*P. falciparum* sodium/hydrogen exchanger), and Pf*mrp* (*P. falciparum* multidrug resistance protein). There is strong evidence that Pf*crt* is associated with chloroquine resistance (18, 32). Pf*mdr1* is involved in mefloquine resistance (15, 31). The evidence of the involvement of Pf*nhe-1* in resistance is compelling but weaker than those for Pf*crt* or Pf*mdr1*. Pf*nhe-1*, which encodes a proton transporter localized to the plasma membrane, may alter quinine activity (4, 17). The evidence for Pf*mrp* being involved in resistance is still debated. However, it seems that Pf*mrp* is associated with decreases in chloroquine and quinine susceptibility (20, 26, 33).

MATERIALS AND METHODS

***Plasmodium falciparum* cultures.** Fifteen monoclonal strains isolated from patients from a wide panel of countries (Brazil, Cambodia, Cameroon, Comoros,

* Corresponding author. Mailing address: Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, 13998 Marseille Armées, France. Phone: 33 4 91 15 01 10. Fax: 33 4 91 15 01 64. E-mail: bruno.pradines@free.fr.

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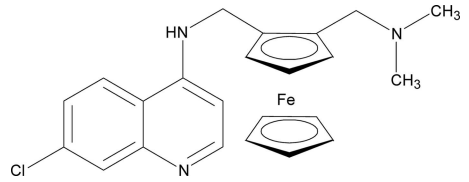

FIG. 1. Chemical structure of FQ {7-chloro-4-[2-*N*,*N*-dimethylaminomethyl)ferrocenylmethylamino]quinoline}.

Djibouti, The Gambia, Honduras, Indochina, Niger, Senegal, Sierra Leone, and Sudan) were maintained in culture in RPMI 1640 medium (Invitrogen, Paisley, United Kingdom) supplemented with 10% human serum (Abcys S.A., Paris, France) and buffered with 25 mM HEPES and 25 mM $NaHCO_3$ (Invitrogen). Parasites were grown in A-positive human blood under controlled atmospheric conditions, which consist of 10% O_2, 5% CO_2, and 85% N_2 at 37°C in 95% humidity.

The strains were synchronized with sorbitol twice before use (23). The susceptibility of each strain to antimalarial drugs was assessed in five independent experiments.

Drugs. FQ base (SR97193) was obtained from Sanofi-Aventis (France). Chloroquine diphosphate (CQ) and quinine hydrochloride (QN) were purchased from Sigma (St. Louis, MO). Monodesethylamodiaquine (MDAQ) was obtained from the World Health Organization (Geneva, Switzerland), and mefloquine (MQ) was obtained from Hoffman-LaRoche (Bale, Switzerland). FQ and CQ were resuspended in water in concentrations ranging between 0.125 and 500 nM for FQ and 5 and 3,200 nM for CQ. QN, MDAQ, and MQ, which were first dissolved in methanol and then diluted in water to obtain final concentration ranges of 5 to 3,200 nM for QN, 1.56 to 1,000 nM for MDAQ, and 3.2 to 400 nM for MQ.

In vitro assay. For in vitro isotopic microtests, 25 μl/well of antimalarial drug and 200 μl/well of the parasitized red blood cell suspension (final parasitemia, 0.5%; final hematocrit, 1.5%) were distributed into 96-well plates. Parasite growth was assessed by adding 1 μCi of tritiated hypoxanthine with a specific activity of 14.1 Ci/mmol (Perkin-Elmer, Courtaboeuf, France) to each well at time zero. The plates were then incubated for 48 h in controlled atmospheric conditions. Immediately after incubation, the plates were frozen and thawed to lyse erythrocytes. The contents of each well were collected on standard filter microplates (Unifilter GF/B; Perkin-Elmer) and washed using a cell harvester (Filter-Mate cell harvester; Perkin-Elmer). Filter microplates were dried, and 25 μl of scintillation cocktail (Microscint O; Perkin-Elmer) was placed into each well. Radioactivity incorporated by the parasites was measured with a scintillation counter (Top Count; Perkin-Elmer).

The drug concentration able to inhibit 50% of parasite growth (IC_{50}) was assessed by identifying the drug concentration corresponding to 50% of the uptake of tritiated hypoxanthine by the parasite in the drug-free control wells. The IC_{50} value was determined by nonlinear regression analysis of log-based dose-response curves (Riasmart; Packard).

Nucleic acid extraction. Total genomic DNA of each strain was isolated by using the E.Z.N.A. blood DNA kit (Omega Bio-Tek, GA) extraction method. RNA of each strain was purified by using the QIAamp Blood Mini kit (Qiagen, Germany).

Pf*crt* single-nucleotide polymorphisms (SNPs). A 1,250-nucleotide-length fragment of the Pf*crt* gene was amplified by reverse transcription-PCR using primers F1sense (5′-TAA TTT CTT ACA TAT AAC AAA ATG AAA TTC-3′) and F1antisense (5′-TTA TTG TGT AAT AAT TGA ATC GAC-3′) and sequenced using primers F2sense (5′-TAG GTG GAG GTT CTT GTC TTG GTA-3′) and F2antisense (5′-TCG ACG TTG GTT AAT TCT CCT TC-3′) (16). Amplifications were performed according to the manufacturer's instructions (Access reverse transcription-PCR system kit; Promega, WI). Sequencing was conducted using ABI Prism Big Dye Terminator v1.1 (Applied Biosystems, CA) cycle sequencing ready reaction kits.

Pf*mdr1* SNPs. Pf*mdr1* was amplified by PCR using primers 5′-TTA CAT TTT ATT TGA TTT TGT GTT G-3′ and 5′-CAT CTT TTC TAG TAT CAT AAT GAA-3′ to amplify codons 86 and 184 and 5′-ACG GGT TTA GTA AAT AAT ATT GTT-3′ and 5′-ATG GGT TCT TGA CTA ACT ATT G-3′ to amplify codons 1034, 1042, and 1246. Amplifications were performed with the Titanium PCR kit (Clontech Ozyme, France) according to the manufacturer's instructions. The amplified fragments were sequenced as previously described.

Pf*mrp* SNPs. PCR amplification followed by sequencing was used to detect SNPs in Pf*mrp* at positions 191 and 437. The primers used for amplification and sequencing were pfmrp-501F (5′-TTT CAA AGT ATT CAG TGG GT-3′) and pfmrp-1409R (5′-GGC ATA ATA ATT GAT GTA AA-3′).

Pf*nhe-1* microsatellite profile. A sequence containing the ms4760 microsatellite described previously (17) was amplified using primers pfnhe-3802F (5′-TT ATTAAATGAATATAAAGA-3′) and pfnhe-4322R (5′-TTTTTTATCATTAC TAAAGA-3′). The amplified fragments were sequenced as previously described.

Statistical analysis. Assessment of standard antimalarial drug cross-resistance with FQ was estimated by determining the coefficient of correlation (r) and coefficient of determination (r^2). The Kruskal-Wallis test or the Mann-Whitney U test was used, when appropriate, to compare equalities of populations for each mutation.

RESULTS

Fifteen *P. falciparum* strains were tested for their in vitro susceptibilities to FQ, CQ, QN, MQ, and MDAQ. FQ had a considerably higher level of activity than did all quinolines tested. The IC_{50} values for FQ ranged from 1.8 to 13.4 nM, with a 5.3 nM mean (standard deviation, ±3.2 nM) (Table 1).

In vitro cross-resistance was measured by the pairwise correlation of IC_{50} values of all 15 strains. Neither FQ and CQ

TABLE 1. In vitro susceptibilities of 15 strains of *Plasmodium falciparum* to FQ, CQ, QN, MQ, and MDAQ

Strain	Origin	Mean IC_{50} (nM) (95% CI)[a]				
		FQ	CQ	QN	MQ	MDAQ
3D7	Sierra Leone	3.4 (3.1–3.8)	21 (18–25)	104 (73–148)	52 (47–58)	21 (15–28)
W2	Indochina	7.6 (6.5–8.8)	485 (377–625)	684 (576–812)	32 (27–37)	146 (113–189)
D6	Africa	5.6 (5.1–6.2)	23 (19–28)	36 (30–42)	59 (50–68)	18 (11–25)
FCM29	Cameroon	6.8 (6.3–7.4)	517 (449–594)	597 (512–696)	28 (21–36)	318 (278–369)
FCR3	The Gambia	1.8 (1.6–2.1)	477 (376–607)	665 (546–811)	30 (26–34)	110 (99–122)
HB3	Honduras	13.4 (11.0–16.2)	18 (11–27)	44 (37–53)	51 (40–65)	35 (29–45)
106/1	Sudan	3.4 (3.0–3.8)	27 (21–33)	66 (60–71)	25 (20–30)	29 (26–33)
IMT Bres	Brazil	3.3 (2.6–4.2)	398 (349–454)	501 (434–579)	33 (28–39)	98 (87–117)
IMT 8425	Senegal	1.9 (1.6–2.2)	29 (26–33)	29 (24–34)	44 (39–50)	26 (21–33)
IMT 10336	Comoros	8.6 (5.8–12.9)	18 (11–27)	220 (180–270)	21 (16–28)	23 (20–27)
IMT K14	Cambodia	7.8 (6.4–9.4)	597 (512–696)	1130 (1042–1225)	33 (28–39)	199 (163–243)
IMT K2	Cambodia	2.2 (1.9–2.6)	531 (441–639)	614 (572–827)	38 (32–45)	91 (78–107)
IMT K4	Cambodia	2.1 (1.7–2.7)	309 (260–367)	1047 (935–1172)	14 (10–18)	146 (113–189)
IMT L1	Niger	4.5 (4.0–5.2)	274 (235–319)	531 (441–639)	38 (32–45)	54 (42–68)
IMT Vol	Djibouti	7.3 (5.6–9.5)	262 (236–292)	422 (377–472)	25 (20–30)	113 (102–125)

[a] Values are mean IC_{50}s of five experiments for each strain. CI, confidence interval.

TABLE 2. Correlation of in vitro responses of 15 strains of *Plasmodium falciparum* to FQ, CQ, QN, MQ, and MDAQ

Drug	Drug partner	r	r^2	P value
FQ	CQ	−0.147	0.0216	0.600
FQ	QN	−0.066	0.0044	0.816
FQ	MQ	−0.011	0.0001	0.970
FQ	MDAQ	+0.086	0.0074	0.760
CQ	QN	+0.756	0.5715	0.001
CQ	MQ	−0.313	0.0980	0.256
CQ	MDAQ	+0.806	0.6496	0.0003
QN	MQ	−0.421	0.1697	0.118
QN	MDAQ	+0.728	0.5300	0.002
MQ	MDAQ	−0.585	0.3422	0.022

($r^2 = 0.0216$) nor FQ and the other quinolines tested were correlated (Table 2). On the contrary, CQ and QN, CQ and MDAQ, QN and MQ, QN and MDAQ, and MQ and MDAQ were significantly correlated.

The following mutations were identified for at least one strain: Pf*crt* M74I, N75E, K76T, A220S, Q271(E/V), N326S, I356T, and I371R; Pf*mrp* H191Y and S437A; and Pf*mdr1* N86Y, Y184F, S1034C, N1042D, and D1246Y (Table 2). Six different ms4760 microsatellite profiles of Pf*nhe-1* were observed (Fig. 2). The numbers of DNNND and DDNHND NHNN repeats on ms4760 ranged from 1 to 4 and 1 to 2, respectively (Table 3).

Polymorphisms in the Pf*crt*, Pf*mdr1*, or Pf*mrp* gene were not associated with *P. falciparum* susceptibility to FQ ($P > 0.386$). On the contrary, in vitro resistance to CQ and reduced susceptibility to QN and MDAQ were significantly associated with mutations in codons 74, 75, 76, 220, 271, 326, 356, and 371 in the Pf*crt* gene ($0.005 < P < 0.05$) and in codons 191 and 437 in the Pf*mrp* gene ($P < 0.007$). Reduced susceptibility to MQ was significantly associated with mutations in codons 74, 75, 76, 220, 271, 326, and 371 in the Pf*crt* gene ($0.017 < P < 0.05$) and in codons 191 and 437 in the Pf*mrp* gene ($P = 0.05$). In addition, in vitro resistance to CQ and reduced susceptibility to QN were significantly associated with mutations in codons 1034 and 1042 in the Pf*mdr1* gene ($P = 0.014$).

The number of ms4760 DNNND repeats in Pf*nhe-1* was not significantly associated with the FQ response ($P = 0.923$), in opposition to those of CQ, QN, and MDAQ ($P < 0.066$).

Statistical analysis was performed for various profiles including ms4760-6 and ms4760-7, which were the most commonly observed profiles. No significant association between FQ or MQ IC_{50} and Pf*nhe-1* ms4760 profiles was established. On the contrary, a significant association was observed for the most frequent profiles (ms4760-6 and ms4760-7) for CQ, QN, and MDAQ ($0.021 < P < 0.049$). Profile 6 was significantly associated with reduced susceptibility to CQ, QN, and MDAQ. Profile 7 was significantly associated with a high level of in vitro resistance to CQ, QN, and MDAQ.

DISCUSSION

FQ, a CQ derivative, is highly active against CQ-resistant *P. falciparum* laboratory strains (13) and against *P. falciparum* strains isolated from infected patients (1, 2, 10, 21, 28, 30). FQ shows good antimalarial and toxicity profiles in rodent malaria models (8). FQ is therefore an interesting candidate for clinical development. FQ is even highly active against parasites with reduced susceptibility to QN, MDAQ, or MQ. FQ is more active than CQ, QN, MDAQ, and even MQ. Encouragingly, we did not find a correlation between FQ and the other quinoline drugs, i.e., CQ, QN, MDAQ, or MQ. These results suggest that no cross-resistance between FQ and CQ, or quinoline antimalarial drugs, exits. These data are in accordance with previous studies, which showed weak coefficients of determination, between 0.096 and 0.127, for correlation between FQ and CQ (2, 21, 28). The potency of FQ against CQ-, QN-, MDAQ-, or MQ-resistant *P. falciparum* strains and the absence of cross-resistance suggest that both drugs have different modes of action or mechanisms of resistance. CQ is believed to act by concentrating in the parasite digestive vacuole and preventing the crystallization of toxic heme in the hemozoin, leading to membrane damage and parasite death (14, 35). Like CQ, FQ forms complexes with hematin in solution and is an inhibitor of β-hematin formation (9). Nevertheless, the absence of cross-resistance between FQ and the other quinolines suggests that FQ may not work exactly as does chloroquine, react with heme and hemozoin differently than the other quinolines, or have a different molecular target.

IC_{50} values for FQ were found to be unrelated to mutations occurring in transport protein genes involved in quinoline antimalarial drug resistance, such as Pf*crt*, Pf*mdr1*, Pf*mrp*, or Pf*nhe-1*. The absence of association with FQ activity and polymorphisms in the Pf*crt* gene is consistent with previous results for Cambodian isolates (10). These data suggest that FQ may not be expelled by transport proteins in quinoline-resistant parasites, possibly as a result of the strong affinity of *P. falciparum* for the iron moiety of the molecule (25). In comparison to CQ, the presence of a ferrocene moiety with a different shape, volume, lipophilicity, effects on basicity, and electrostatic profile dramatically modifies the pharmacological behavior of the parent drug (9). Therefore, FQ appears to present reduced affinity for the transporters involved in the resistance to CQ and quinoline drugs. This may partially explain the high level of activity of FQ against multidrug-resistant *P. falciparum* parasites. This is consistent with results that indicate that the ability of mutant PfCRT to confer CQ resistance is precisely

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ms4760-1    KKKKISGSNNDNNDNNNDDNNND----------NHNDDKNNKNDDDNHNDNHNNDDDNHNDNHNNDDDNNNDNHNDDDNNSSHYNKEKE
ms4760-2    KKKKISGSNNDNNDNNNDDNNND----------DKNNKNDDDNHNDNHKNDDDNHNDNHNNDDDNNNDNHNDDDNNSSHYNKEKE
ms4760-3    KKKKISGSNNDNNDNNNDDNNND----------DKNNKNDDDNHNDNHNNDDDNHNDNHNNDDDNNNDNHNDDDNNSSHYNKEKE
ms4760-5    KKKKISGSNNDNNDNNNDDNNNDDNNNDDNNNDNHNDDKNNKNDDDNHNDNHNND----------DDNNNDNHNDDDNNSSHYNKEKE
ms4760-6    KKKKISGSNNDNNDNNNDDNNND----------NHNDDKNNKNDDDNHNDNHNND----------DDNNNDNHNDDDNNSSHYNKEKE
ms4760-7    KKKKISGSNNDNNDNNNDDNNNDDNNND-----NHNDDKNNKNDDDNHNDNHNND----------DDNNNDNHNDDDNNSSHYNKEKE
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FIG. 2. Sequences of Pf*nhe-1* microsatellite ms4760 detected among the 15 studied *P. falciparum* strains. Profiles 1 to 7 were previously described (16).

TABLE 3. Pf*crt*, Pf*mdr1*, Pf*mrp*, and Pf*nhe-1* polymorphisms in *Plasmodium falciparum* strains[a]

Strain	Amino acid encoded by:																					No. of Pf*nhe-1* microsatellite ms4760 DNNND repeats	No. of Pf*nhe-1* microsatellite ms4760 DDNHNDNHNN repeats	Mutation at Pf*nhe-1* microsatellite ms4760[a]	Pf*nhe-1* microsatellite ms4760 profile
	Pf*crt* codon:											Pf*mdr1* codon:						Pf*mrp* codon:							
	72	74	75	76	97	144	148	194	220	271	326	333	356	367	371	86	184	1034	1042	1246	191	437				
3D7	C	M	N	K	H	A	L	I	A	Q	N	T	I	G	**R**	N	Y	S	N	D	H	S	1	2	**K**	2
W2	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	**T**	G	I	**Y**	Y	S	N	D	**Y**	A	2	2	N	1
D6	C	M	N	K	H	A	L	I	A	Q	N	T	I	G	**R**	N	Y	S	N	D	H	S	1	2	N	3
FCM29	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	3	1	N	7
FCR3	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	2	1	N	6
HB3	C	M	N	K	H	A	L	I	A	Q	N	T	I	G	**R**	N	**F**	S	N	D	H	S	4	1	N	5
106/1	C	**I**	**E**	K	H	A	L	I	**S**	**E**	**S**	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	2	1	N	6
IMT Bres	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	2	1	N	6
IMT 8425	C	M	N	K	H	A	L	I	A	Q	N	T	I	G	**R**	N	Y	S	N	D	H	S	1	2	**K**	2
IMT 10336	C	M	N	K	H	A	L	I	A	**E**	N	T	I	G	**R**	N	Y	S	N	D	H	S	1	2	N	3
IMT K14	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	**T**	G	I	N	**F**	**C**	**D**	**Y**	**Y**	A	3	1	N	7
IMT K2	C	**I**	**E**	**T**	H	A	L	I	**S**	**V**	**S**	T	**T**	G	I	N	**F**	**C**	**D**	D	**Y**	A	3	1	N	7
IMT K4	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	**T**	G	I	N	Y	**C**	**D**	D	**Y**	A	3	1	N	7
IMT L1	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	N	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	2	1	N	6
IMT Vol	C	**I**	**E**	**T**	H	A	L	I	**S**	**E**	**S**	T	I	G	I	**Y**	Y	S	N	D	**Y**	A	2	1	N	6

[a] Polymorphism types are detailed in Fig. 2. Boldface type indicates point mutations.

configured for CQ (22). Resistance was rapidly lost following subtle structural modifications of the basic diethylamino side chain linked to the 4-aminoquinoline ring structure. Cross-resistance was clearly evident with analogs that varied by only a single CH_2 group and absent when two CH_2 groups were removed or six were added (22). In addition, *Dictyostelium discoideum* transformants expressing the CQ resistance phenotype PfCRT were not able to expel piperaquine, a bisquinoline analog of CQ (27). The absence of an interaction of FQ with PfCRT suggests that the phenotypic response to FQ would not be modified by resistance reversers such as verapamil. Nevertheless, the effects of reversers on aminoquinoline analogs are still debated. Verapamil did not affect the relative piperaquine response in *D. discoideum* transformants expressing the CQ resistance phenotype PfCRT at concentrations that completely reverse CQ resistance (27), while desipramine could reverse resistance to bisquinoline WR268,668 (3). The ability of verapamil to enhance the activity of a drug is inversely related to the log *D* of this drug (19). In addition, no resistance of *P. falciparum* to FQ has been found in vitro in either patient isolates or laboratory-adapted strains under drug pressure (11).

In conclusion, FQ is highly active against parasites with reduced susceptibility to QN, MDAQ, or MQ. No cross-resistance between FQ and CQ, or quinoline antimalarial drugs, exists. IC_{50} values for FQ were found to be unrelated to mutations occurring in transport protein genes involved in quinoline antimalarial drug resistance, such as Pf*crt*, Pf*mdr1*, Pf*mrp*, or Pf*nhe-1*. The present results justify clinical trials of FQ in multidrug resistance areas. A phase II study is now in progress in Gabon.

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